4 Role of environment in CFA

4.1 Socioeconomic status and orofacial clefts

The investigation of the relationship between socioeconomic status and the prevalence of various health outcomes has provided important clues as to etiology. For example, the observation of an increasing risk of neural tube defects with decreasing socioeconomic status was one of the clues to a dietary hypothesis for these defects (Elwood and Colquhoun, 1992).

Little attempt has been made to investigate whether the risk of orofacial clefts varies by socioeconomic status. Womersley and Stone (1987) examined the prevalence at birth of orofacial clefts within Greater Glasgow (Scotland) during the period 1974-1985, according to housing and employment characteristics recorded in the 1981 census. The highest rates were observed in areas with high proportions of local authority housing with young families, high unemployment and a preponderance of unskilled workers, whereas the lowest rates were found in affluent areas with high proportions of professional and non-manual workers in large owner-occupied or high-quality housing. Most of this pattern was accounted for by CP, with less variation in CL/P.

A variety of different indicators of socioeconomic status have been developed (Liberatos et al., 1988). In an international context, it seems appropriate to use one that is specific to the local area, and one that can be compared between countries, e.g. years of schooling. As socioeconomic status can be difficult to determine at the level of the individual, especially for women, there has been increasing interest in developing, and using, area-based measures of material deprivation as a proxy for socioeconomic status (Townsend,1987; Carstairs and Morris, 1990).

4.1.1 Orofacial clefting, socioeconomic status, nutrition and dietary supplements

Socioeconomic status may have a number of associated variables contributing to the explanation, such as nutrition, smoking, alcohol, illnesses and infections. These factors tend to have been studied retrospectively in some parts of the world and such studies are now being carried out prospectively in Denmark and Norway with regard to reproductive outcome. Other aspects of nutrition not well studied are the effects of obesity/starvation and it may be useful in future studies to record height and weight to get a measure of body mass index in relation to orofacial clefts.

4.1.2 Conclusions

The evidence for prevalence of OFC being greater in the lower socioeconomic classes remains equivocal, the less well-developed countries having a greater proportion of the population in the lower socioeconomic classes.

The overall conclusion is that socioeconomic status and OFC are not well studied. One of the barriers to investigation of the role of socioeconomic status in orofacial clefting is that common criteria for the description of low socioeconomic status do not exist and, in those studies where socioeconomic status or social class have been examined, different criteria have been used, thus making valid inter-centre comparisons impossible.

4.2 Nutrition and orofacial clefts: general issues

There is considerable interest in the effects of maternal nutrition, during the peri-conceptional period, on the occurrence of several types of congenital anomalies. This interest has been stimulated by the finding in a randomized controlled trial that maternal peri-conceptional folic acid supplementation reduces the recurrence risk of neural tube defects (MRC Vitamin Study Research Group, 1991). The role of maternal peri-conceptional vitamin status is now being debated in relation to:

- orofacial clefts (Tolarova and Harris, 1995; Shaw et al., 1995a; Czeizel, 1996; Hayes et al., 1996);
- limb defects (Shaw et al., 1995b);
- conotruncal heart defects (Shaw et al., 1995b; Botto et al., 1996; Scanlon et al., 1998);
- and urinary tract malformations (Li et al., 1995; Czeizel, 1996).

4.2.1 Variation in diet

BOX G

Worldwide variation in diet

Dietary patterns vary greatly between different parts of the world. In rural areas of developing countries, diets may depend solely on what a family or local community produces. As the use of cash is extended, a greater variety of foods becomes available in local markets or shops. In economically developed societies and in urban areas in developing countries, diets are influenced not only by food supplies grown and processed locally but also by those available nationally and internationally (World Cancer Research Fund, 1997).

The diets typically consumed in rural parts of Africa, Asia, Latin America and Oceania often rely on one or two staple cereal foods. In China, India and other low-income countries of Asia, cereals tend to be dominant. Rice dominates in Asia, wheat in North Africa, maize in Latin America, and maize and starchy roots in sub-Saharan Africa (World Cancer Research Fund, 1997).

As countries develop economically, consumption of the dominant staple cereal foods declines. There is a fall in the overall consumption of foods of plant origin and replacement with increasing amounts of foods of animal origin, notably meat, meat products and dairy products. Sugar consumption also tends to increase. Compared with the diets of less developed societies, such diets are lower in fibre and other bioactive compounds found in foods of plant origin. An ever-increasing proportion of food in industrialized societies is processed (World Cancer Research Fund, 1997).

Within some of the most economically-developed countries, this process has slowed and, for some population subgroups, has reversed. For example, in some northern European countries and within North America, there is a trend towards increasing consumption of vegetables and fruits, and decreasing consumption of red meat, fat, full-fat milk, other dairy products and sugar in the form of sucrose (World Cancer Research Fund, 1997).

4.2.2 Diet in pregnancy

During the 40 weeks of pregnancy, an average 12.5-15.0 kilograms are gained (Lederman, 1991). This may be lower in populations with chronic food shortage, or when weight-gain limitation is recommended, as was the case in the United States in the 1960s. In view of the weight gain during pregnancy, an increased food intake would be expected. There have been few studies of intake changes during pregnancy in the same women. The available studies suggest some increased intake in mid-gestation (Lederman, 1991; Brown and Kahn, 1997) but the relationship of this to

intake prior to pregnancy, or around the time of conception, is unclear. In a study of about 550 women in Minnesota (United States) recruited prior to pregnancy and followed at monthly intervals until 6-8 weeks postpartum, the peak increase in total energy intake, and peak decrease in energy expenditure, occurred within the first nine weeks of pregnancy (Brown and Kahn, 1997). Postpartum energy intake declined and energy expenditure increased.

About 50% of pregnant women experience nausea or vomiting during early pregnancy (Kullander and Kallen, 1976; Klebanoff et al., 1985). It appears that women experiencing nausea and vomiting tend to cut down or stop their consumption of alcohol, coffee, tea and other potentially harmful beverages, and also stop smoking (Hook, 1976; Golding, 1986), but the effects on maternal diet appear to have been little documented. It has been suggested that elevated estrogen levels early in pregnancy are the main cause of vomiting, but the evidence is inconclusive (Zhang and Cai, 1991).

Assessment of dietary intake is problematic

4.2.3 Biochemical markers and gene/nutrient interaction

Assessment of dietary intake is problematic. The most established method in nutritional epidemiological investigation of chronic diseases is the food frequency questionnaire (FFQ) in which the primary aim is to obtain a relative ranking of subjects in terms of their reported intake, rather than to determine their absolute intake. Misclassification is recognized as a major problem.

In addition to food frequency data, it is also useful therefore to have biochemical markers of nutrition but, because metabolism is under genetic control, these measures are not the same but complementary. One promising area for future research in the influence of socioeconomic status and nutrition in OFC is the examination of genetic polymorphisms which effect nutrient metabolism, e.g. MTHFR and folate receptors, with study designs aimed to examine gene/environment interaction. While these hypotheses are generated on the basis of biological plausibility, there might well be gene/environment interactions with no apparent biological plausibility, such as reports of interaction between TGFα (transforming growth factor) and multivitamins, and TGFα and smoking. In developing countries there is a need to design FFQs and collect data on nutrition in close consultation with the local indigenous people. There may be a tendency for FFQs to exclude important groups of food that are being consumed. It is also important to realize that people eat foods and not nutrients – which makes it challenging to identify the effects of specific nutrients.

4.2.4 Conclusions

In planning or appraising a study of nutritional epidemiology, in addition to the usual considerations of bias, confounding and chance, important criteria are:

(1) use of a validated dietary instrument that estimates total energy intake;

(2) appropriate adjustment for total energy intake in statistical analysis;

(3) whether any biological markers used are appropriate for the hypotheses under test, and the possible effect of their use on participation rates.

The importance of multi-centre collaborative efforts in looking at diet and nutrition is the broad range of exposure that will reduce the impact of misclassification. However, it is recognized that this is also likely to introduce more heterogeneity.

4.3 Folic acid: nutritional biochemistry and orofacial clefts

4.3.1 Folic acid in reproduction

The terms "folic acid" and "folate" both refer to the same vitamin, whereby folate is the polyglutamate natural form and folic acid is the monoglutamate synthetic form. Adequate maternal folate status is crucial to all stages of pregnancy from conception to delivery. Folate nutrition seems to have a dual role in determining pregnancy outcome. One of these is the long-established role in fetal maturation that may place a requirement for supplementation to prevent maternal anaemia in late pregnancy (Scott and Weir, 1998). The other is the newly-perceived role in the prevention of congenital defects during early embryonic development.

4.3.2 Maternal folic acid deficiency

Peri-conceptional folic acid supplementation can prevent the majority of neural tube defects (NTDs) (MRC Vitamin study, 1991; Czeizel and Dudas, 1992). The mechanism does not seem to be a correction of maternal clinical folate deficiency (Kirke et al., 1993). Nevertheless, there is a strong inverse relationship between a mother's early-pregnancy red cell folate concentration and her risk of having an NTD-affected birth (Daly et al., 1995). This, along with other genetic and environmental evidence, indicates that a complex interaction of folate-related nutritional and genetic influences underlie the etiology of NTDs. The evidence of folate

involvement with other congenital defects is not as strong, but is nevertheless encouraging (Finnell et al., 1998). Early trials using vitamin supplementation to reduce recurrence of orofacial clefting were inconclusive. Many of these studies were small, non-randomized and the treatment preparation was a multivitamin containing folic acid. Other evidence suggesting a link between folate and orofacial clefts included positive associations between clefts and (a) maternal use of anticonvulsants and other known folate antagonists, or (b) maternal cigarette and alcohol abuse (both of which interfere with folate status). In addition, some animal studies showed that feeding folate-deficient diets or administration of antifolate drugs to pregnant rats could induce craniofacial abnormalities in rat embryos. It has been suggested that maternal folic acid supplementation plays a role in the prevention of non-syndromic orofacial clefts, i.e., cleft lip with or without cleft palate (CL/P). Using a case-control design, Wong et al. (1999) investigated vitamin-dependent homocysteine metabolism in 35 mothers with non-syndromic orofacial cleft offspring and 56 control mothers with non-malformed offspring. A standardized oral methionine-loading test was performed, in which fasting and afterload plasma total homocysteine, serum and red-cell folate, serum vitamin B12 and whole-blood vitamin B6 levels were determined. The test showed that both fasting ($p < 0.01$), as well as afterload ($p < 0.05$) homocysteine concentrations, were significantly higher in cases compared to controls.

Early trials using vitamin supplementation to reduce recurrence of orofacial clefting were inconclusive

Hyperhomocysteinemia, defined by a fasting and/or afterload homocysteine concentration above the 97.5th percentile, was present in 15.6% of the cases and in 3.6% of controls (odds ratio (OR) 5:3, confidence interval (CI) 1.1 to 24.2). The median concentrations of serum ($p < 0.01$) and red-cell ($p < 0.05$) folate were significantly higher, and vitamin B6 concentrations appeared to be significantly lower ($p < 0.05$) in cases compared with controls. No significant difference was observed between groups for vitamin B12. These preliminary data offer evidence that maternal hyperhomocysteinemia may be a risk factor for having non-syndromic orofacial cleft offspring. In a more recent study among Irish orofacial cleft cases an increased prevalence of a genetic variant of a folate-related enzyme, previously shown to cause increased risk of NTDs, was found (Mills, 1999; Shields et al., 1999). Homozygosity for this common polymorphism occurs in between 5 to 25% of populations worldwide. The variant phenotype expresses reduced enzyme activity and adversely affects folate status (Molloy et al., 1997). This study recognizes the possibility of population differences in genetic susceptibility, and the need for research on gene/environment interaction.

4.3.3 Folic acid metabolism

It would clearly be unethical at this point to conduct a randomized placebo-controlled trial of folic acid and clefts, given the proven benefit of folic acid in preventing NTDs. Thus the identification of a role for folate or indeed other nutrients will have to be pursued by other means. In other words, it will be necessary to study genetic, nutritional or environmental markers of risk. A randomized controlled trial of different doses would be theoretically possible; there are questions with regard to ethics in study design which are discussed in more detail in Section 7.4 below. From a mechanistic point of view there are good reasons why aberrations in folate metabolism might cause congenital abnormalities. Within the cell, the overall function of the folate co-factors is to accept 1-carbon units from several sources and donate them to other molecules in a variety of enzyme reactions. These 1-carbon units are required for the production of purines and pyrimidines for DNA synthesis and to maintain a supply of methyl groups for the methylation of DNA, proteins, neurotransmitters, etc. (Scott and Weir, 1998). Early embryonic development requires extensive DNA synthesis. An adequate capacity to methylate DNA is crucial in the control of gene expression and thus would be an essential component of cell differentiation and development. Thus, genetic variations in folate-related enzymes, altered nutrition or environmental factors influencing folate status could all be considered to be potential risk factors for congenital malformations and candidates for research into the underlying causes of craniofacial anomalies.

4.3.4 Etiologic heterogeneity in OFC

There are, however, several difficulties associated with this approach. The first of these is etiologic heterogeneity of orofacial clefts, apart from the 20% or so that are syndromic due to specific mutations. It is quite possible that a specific fraction of orofacial clefts are related to folic acid or other multivitamins, but these are submerged under a sea of non folate-related defects. Some of these etiologies may be responsive to folate or other nutrients, others may not, making it difficult to find positive effects. Secondly, any potential genetic or biochemical markers of moderate risk may be difficult to detect unless the majority of syndromic cases can be ascertained and excluded from study sets. Thirdly, it will be important to have the capability of monitoring the nutritional or biochemical biomarkers that may be affected by new polymorphisms which are discovered in candidate genes. This means that a system involving collection of blood and perhaps immortalized cells should be set in place for future analyses. However, the logistics of such an undertaking would need to be carefully considered so that the task is comprehensive enough

to be effective without breaking the back of an entire research endeavour. Finally, while conclusive evidence exists for a specific protective role of folate in prevention of NTDs, this is not the case for orofacial clefts. The present indications of nutrient protection are derived from multivitamin preparations and not just folate.

4.3.5 Research strategy to deal with data gaps

In terms of approaches one could take to improve our level of evidence, there are many problems in carrying out good controlled studies to look at the role of folate and one of the biggest obstacles to progress is the heterogeneity of the study population. To minimize the problem in identifying folate-related defects, it will be essential to carefully categorize samples by type of defect, to identify (and exclude) syndromic cases where possible, and to control methodologic and demographic parameters which might confound biochemical and genetic analyses. In terms of identifying factors that influence folate status, genetic influences might play a major role. This was highlighted in a study of mono- and di-zygotic twins (Mitchell, 1997) that suggested that as much as 46% of the variance in red-cell folate concentrations might be attributable to additive genetic effects.

Most studies find that food folate intake does not have a high correlation with red-cell folate levels

4.3.6 Uses and limitations of FFQ data as an alternative to blood samples

Misclassification is undoubtedly a problem with FFQs but surprisingly few biomarkers give a clear picture of nutritional intake. The inter-correlation between nutrients is also a problem for either FFQs or bio-chemical measurement. In the case of folate, at least, FFQs alone are very flawed, particularly when carrying out retrospective studies – most studies find that food folate intake does not have a high correlation with red-cell folate levels (correlation approximately 0.4). The chance of finding a folate-related effect on data derived from FFQs alone would have to be very small; nevertheless, the precise and detailed information requested by these questionnaires may possibly give one a false sense of security in the data. There are also practical difficulties with food tables in field conditions – particularly in assessing poorly nourished people in developing countries.

The alternative or complement to questionnaires for nutrient measurement is blood sampling and carrying out case-control studies on nutrient levels (or bio-markers such as homocysteine), provided disease status does not affect nutrient levels. Having overcome the logistics of sampling, there is an important issue in deciding from whom to collect blood (case, mother, father, or controls) and when the most appropriate

time to take a blood sample would be. While this was not resolved it was, however, recognized that the major problem of measurement bias in biochemical analyses and inter-laboratory differences in methodology to measure blood levels of folates could be overcome by centralizing and standardizing sample analyses in a reputable laboratory, using another laboratory to ensure quality control.

4.4 Other specific nutrients and orofacial clefts[1]

4.4.1 Vitamin B-6

Vitamin B-6 has been shown to protect against teratogen-induced clefts in many animal studies. Vitamin B-6 is the generic term for 3-hydroxy-2-methylpyridine derivatives that have the biological activity of pyridoxine. This vitamin plays many vital roles in amino acid metabolism, including transamination and decarboxylation reactions, and is the coenzyme in the degradation of homocysteine; there are thus many potential pathways in which vitamin B-6 protects against orofacial clefts. Vitamin B-6 deficiency alone was demonstrated to cause cleft palate and other birth defects in mice (Davis et al., 1970). Miller (1972) demonstrated that dietary vitamin B-6 also prevented the induction of clefts by vitamin A excess, cyclophosphamide, and beta-aminoproprionitrile; hence the role of vitamin B-6 in cleft prevention may be complex and involve several different mechanisms.

Despite the extensive investigation of the role of vitamin B-6 in animal models of clefting since the 1950s, there is little information on the relevance of vitamin B-6 to clefts in humans. Use of anti-nausea medications has been associated with a reduced risk of congenital heart defects in the Atlanta Birth Defects Case-Control Study (Erickson, 1991), and vitamin B-6 may have a role in this pathway *(see also Section 7.3.2)*.

4.4.2 Vitamin A

In experimental animals, vitamin A has been described as a "universal teratogen" (Schardein, 1993). The possible teratogenicity of dietary and supplementary vitamin A intake in the peri-conceptional period or early pregnancy in humans is controversial (Rothman et al., 1995; IARC Working Group, 1998; Miller, 1998). The debate has focused in particular on anomalies of structures derived from cranial neural crest cells, of which orofacial clefts are the most common type. There are considerable differences in the minimum teratogenic dose between species (IARC Working Group, 1998). The identification of genetic polymorphism at retinoic acid effector loci (RARA, AA7, MSX1) in

[1] See also Section 7.3.

humans raises the question as to whether there are inter-individual variations in susceptibility to the possible teratogenic effects of high intakes of vitamin A *(see also Section 7.3.4)*.

4.4.3 Zinc

Studies associating maternal zinc nutriture to the risk of orofacial clefts in humans are extremely limited. Only one study has been conducted to evaluate the association by independently analysing the risk of orofacial clefts from other malformations. In addition, there have been a few investigations involving a limited number of cases of orofacial clefts, where no meaningful statistical analysis was possible (Flynn et al., 1981; Soltan and Jenkins, 1982; Stoll et al., 1999).

4.5 Lifestyle, occupational and other environmental factors in orofacial clefting

4.5.1 Cigarette smoking

BOX H

Maternal cigarette smoking in pregnancy [2]

Maternal cigarette smoking during pregnancy has long been associated with a moderate increase in the risk of orofacial clefts (Andrews and McGarry, 1972; Kelsey et al., 1978; Khoury et al., 1987; Shaw et al., 1996; Kallen, 1997; Werler et al., 1990; Ericson et al., 1979; van den Eeden et al., 1990), although some studies have not confirmed such an association (Evans et al., 1979; Shiono et al., 1986; Malloy et al., 1989; Hwang et al., 1995). A recent meta-analysis of published literature (Wyszynski et al., 1997) produced a summary:

- OR of 1:29 (95% CI 1.18 to 1.42) for CL/P associated with maternal smoking during pregnancy; and
- OR 1:32 (CI 1.10 to 1.62) for CP.

As in many epidemiological studies on birth defects showing weak effects, several potential methodological problems can obscure a true causal association (Khoury et al., 1992). For instance, several studies have not considered the following separately: CL/P and CP; isolated and multiple forms (Khoury et al., 1989). In most studies, there is no evidence of a linear dose-response relationship between cigarette consumption and risk of orofacial clefts. However, if such an association were confirmed, cigarette smoking might account for as much as 20% of orofacial clefts in the general population (Khoury et al., 1989). Parallel investigation of genetic susceptibility and of gene/environment interaction in relation to smoking would also be of interest.

[2] See also Section 7.2.1.

4.5.2 Alcohol drinking[3]

Heavy alcohol drinking during pregnancy is known to alter embryonic development, and cleft palate has been described as an associated defect in 10% of severe cases of fetal alcohol syndrome (Lemoine, 1992). An increased risk of CL/P specifically was found in association with a heavy intake of five drinks or more per day (OR=3:0; 95% CI 1.1 to 8.5), a category which concerned only 0.5% of control mothers (Werler et al., 1991). In a recent study in the United States (Iowa), maternal consumption of more than 10 drinks per month was associated with increased risks for isolated CL/P (OR=4:0; 95% CI 1.1 to 15.1) and isolated CP (OR=1:8; 95% CI 0.3 to 12.1), statistically significant only for CL/P (Munger et al., 1996). Paternal drinking was not associated with orofacial clefts (Savitz et al., 1991). One problem in the quantitative interpretation of the few studies on maternal alcohol consumption and orofacial clefts is the wide range of consumption across studies, in which similar effects can be found for a consumption of 5 drinks per day in 1 study and 10 drinks per month in another. In a systematic review presented at the WHO consensus meeting in Utah (May 2001), Little noted that the interpretation of the relationship between alcohol and orofacial clefts may be complicated by publication bias. In a number of studies of smoking, alcohol has been considered as a potential confounder, but no primary results relating to alcohol have been presented.

4.5.3 Other environmental risk factors

There is an association between orofacial clefts and epilepsy, but some controversy about whether it is the disease or the treatment with anti-epileptic drugs (AEDs) such as phenytoin or phenobarbital that is important. It has been estimated that the risk of CL/P among a new-born of a treated epileptic mother may be as high as 1%, i.e. about 10 times the population average (Dravet et al., 1992; Johnston and Bronsky, 1995). In general, as far as it is possible to separate effects of disease and therapy, risks associated with treatment with AED (especially polytherapy) are higher than those associated with disease alone (not treated) (Abrishamchian et al., 1994; Wyszynski, 1996). Among all AEDs, phenytoin has been more specifically associated with the risk of orofacial clefts (Johnston and Bronsky, 1995; Dravet et al., 1992), and the folic acid antagonistic effect is a possible mechanism *(see below)*. To help resolve this, examination of familial aggregration and the rate of clefts in the offspring of men with epilepsy can be undertaken.

[3] See also Section 7.2.2.

4.5.4 Other illnesses and medications

A number of other environmental factors may influence the occurrence of orofacial clefts:

- **Viruses:** acute viral infections and cold have both been reported as having associations with clefts (e.g. Czeizel and Hirschberg, 1997), and there may be confounding by hyperthermia.
- **Folic acid antagonists:** possibly a factor in CLP but not CP (Hernandez-Diaz et al., 2000).
- **Benzodiazepines:** some studies show an increase in risk – retrospectively, but not prospectively.
- **Corticosteroids:** some studies show an association, but the difference between topically- and systemically-applied corticosteroids requires further investigation.
- **Retinoids and tretinoin:** known teratogens in animal experiments, but there is little evidence of their association with orofacial clefts.

4.5.5 Occupational exposures

Pesticides/herbicides, water contaminants and occupational exposures have been examined in relation to OFC. Registry data (Ericson et al., 1979; Hemminki et al., 1981) and large-scale studies (McDonald et al., 1988) have suggested associations between orofacial clefts and maternal occupation (health workers, the repair-services industry, industrial trade or agriculture). Subsequent studies among health workers have not confirmed an increased risk (Matte et al., 1993). Maternal occupational exposure to solvents has been related to orofacial clefts in the early study by Holmberg et al. (1982), and subsequent studies in France (Cordier et al., 1992; Laumon et al.,1996) and Europe (Cordier et al., 1997). Teratogenesis with trichloroethylene and tetrachloroethylene in water has been suggested and associations with farming work have indicated a possible role of pesticides, confirmed in some published studies (Gordon, 1981; Thomas et al., 1992; Nurminen et al., 1995) but not in others (Shaw et al., 1999). It is important to specify the study period as this may affect the type and intensity of exposure, and the measures in place to protect against potential adverse effects of exposure (e.g. regulations about use of respirators, etc.).

Occupations of the father in the printing industry, as a painter (Erickson et al., 1979), motor vehicle operator (Olshan et al., 1991), fireman or farmer (Schnitzer et al., 1995) have been associated with an increased risk of orofacial clefts.

4.6 Conclusions

- Main gaps in knowledge are in the examination of co-teratogens and gene/environment interaction – for example: with alcohol in fetal alcohol syndrome (FAS) are there co-teratogens such as folate deficiency, and is there a threshold beneath which alcohol is safe? and with alcohol drinking, is there an indication of a dose response in terms of risk, with greater than 500 ml per day showing a significant association?
- Smoking, alcohol, epilepsy, certain medications and environmental factors may explain a small but appreciable portion of birth defects.
- It is important to be able to differentiate the exposure and the genetic predisposition so that those at risk can be identified and selectively counselled.
- General advice regarding alcohol and smoking in relation to disease tends to be ineffective in achieving significant changes in behaviour. Novel strategies surrounding birth defects may achieve better results. However, one major issue in the reporting of associations with exposures is the distinct possibility of publication bias in the literature.